TRUDEAU (E. L.) & BALDWIN (E. R.)

A RÉSUMÉ OF EXPERIMENTAL STUDIES ON THE PREPARATION AND EFFECTS OF ANTITOXIC SERUM IN TUBERCULOSIS.

BY

E. L. TRUDEAU, M.D.,

AND

E. R. BALDWIN, M.D.

(*From the Saranac Laboratory for the Study of Tuberculosis, Saranac Lake, N. Y.*)

REPRINTED FROM THE
TRANSACTIONS OF THE ASSOCIATION OF AMERICAN PHYSICIANS.
1898.

A RÉSUMÉ OF EXPERIMENTAL STUDIES ON THE PREPARATION AND EFFECTS OF ANTITOXIC SERUM IN TUBERCULOSIS.

By E. L. TRUDEAU, M.D., and E. R. BALDWIN, M.D.

(From the Saranac Laboratory for the Study of Tuberculosis, Saranac Lake, N. Y.)

The brilliant and beneficent results which have followed the discovery and use of the diphtheria and tetanus antitoxic serum have stimulated similar research in many of the infectious diseases. But the work in other fields has led to results which, in their practical phases at least, are as yet disappointing.

Among the infectious diseases to which attention was early turned in the studies on artificial immunity, tuberculosis is one which presents aspects at once attractive and forbidding. Although workers in the field have not been idle, it must be conceded that the attempts to provide a curative or antitoxic serum for tuberculosis have not thus far been such as to inspire the confidence of those fitted by technical training and clinical experience to judge.

Simultaneously with the development of tetanus and diphtheria antitoxin, even earlier (1888), Hericourt and Richet[1] reported favorable results in conferring immunity to tuberculosis in rabbits by injections of the serum of immunized dogs. Unfortunately, clinical tests of the efficacy of this serum when applied to human tuberculosis did not prove at all encouraging. Nevertheless, the efforts of experimenters to advance existing knowledge of the toxic products of the tubercle bacilli, to produce an artificial immunity against tuberculosis in animals, and to obtain an antitoxic serum for this disease, have been steadily carried on, and some light has been thrown on this all-important and complex problem.

One of us (Trudeau)[2], [3] has been engaged in attempts at producing

immunity to tuberculosis since 1891; the present paper, however, includes only our studies with serums, which were commenced in 1894. While confessing our disappointment at the outcome of most of these experiments, we yet feel warranted in presenting them, because they seem to us to indicate some interesting phases of work in tuberculosis and the care needed to make safe deductions from laboratory experiments.

The efficacy of any antitoxin serum and the ease with which it is obtainable would seem, judging by analogy, to be in direct ratio to the degree of toxicity possessed by the poison against which protection is sought. For this reason, to produce and demonstrate antitoxic properties in serums would, *à priori*, seem to be more difficult in a disease so chronic as tuberculosis on account of the low dergee of toxicity of its products. The antituberculous serums which we have produced by our own methods, the antitoxic power of which could be proved experimentally on animals, and our tests of the antitoxic properties in the serums produced by others, have shown only slight potency for any of them up to the present time. Tuberculosis does not belong to that class of infectious diseases which kill by acute toxæmia, but to the class to which syphilis, actinomycosis, and leprosy also belong, and which destroy life, not only by the chronic and long-continued systemic poisoning they produce, but by the pathogenic changes brought about through the localization and growth of the germs in organs necessary to life.

Koch's[4] assumption seems plausible, that immunity to the toxic products of the tubercle bacillus does not necessarily imply immunity to tuberculosis, and a serum which would neutralize the toxic effects of tuberculin may not prevent the growth of the tubercle bacillus in the tissues and its destructive action on the organs of the body. An efficacious serum for this disease would probably require, therefore, to possess not alone antitoxic but also germicidal properties, or at least the power to excite the organism to germicidal activity. Bactericidal properties have been claimed for certain serums by careful observers, but the specificity of any such action is open to question; and the existence of any demonstrable degree of germicidal power in antituberculous serums has not been confirmed generally by other observers.

It is not at all certain that, even if bacterial immunity could be produced by any method, the serum of animals possessing this immu-

nity would necessarily be either antitoxic or germicidal. Indeed, one of us (Trudeau) has succeeded in producing a marked degree of immunity in rabbits by preventive inoculation of living cultures of tubercle bacillus attenuated by prolonged cultivation; and yet the serum of these animals which had resisted a subsequent virulent inoculation proved to have but slight, if any, antitoxic power, and did not seem to influence to an appreciable degree the course of the disease in tuberculous guinea-pigs.

Notwithstanding the meagre results obtained in animal experiments, a good deal of clinical evidence as to the value of serum treatment in tuberculosis has been presented, which, however, is not sufficiently encouraging to be convincing; since this disease runs, without any specific treatment, so varied and erratic a course. The clinical evidence has not been considered in this research, and must be judged by itself. These studies have been entirely confined to such experimental proof of the presence of curative and antitoxic properties in serums as could be obtained by laboratory methods.

The nature of the bacterial poisons used in injecting animals with a view to producing curative serums is probably of vital importance to the success of such attempts.

When we began this work the observations of Hammerschlag,[5] Koch,[6] Proskauer and Brieger,[7] Hueppe and Scholl,[8] Weyl,[9] Hericourt and Richet,[10] Crookshank and Herroun,[11] Richet,[12] Bâbes,[13] Zuelzer,[14] Klebs,[15] Hahn,[16] Kühne,[17] Hoffman,[18] Matthes,[19] de Schweinitz,[20] and our own, agreed in the main as to the presence of poisonous albuminous substances in cultures of tubercle bacillus which were products of the germ-growth and had chemical reactions like the albumoses, albuminates, and nucleo-proteids,* and all producing the characteristic physiological action of tuberculin. It is to be noted that large quantities of the filtered culture-fluid are borne by healthy animals without immediate toxic effects, while quite small doses may produce death in tuberculous animals within a few hours.

No material addition to our knowledge of these culture-products has been published to the present time. Behring[21] has recently reported the separation of a more active poison than hitherto obtained.

* A single phosphorus determination, kindly made for us by Prof. R. H. Crittenden, of the pure proteid obtained from cultures on synthetic media (containing no peptone nor albuminoid) gave a content of 1.52 per cent. This indicated the presence of considerable nucleo-proteid produced by the tubercle bacillus.

De Schweinitz and Dorset[22] have prepared small quantities of a necrotizing substance. More recently Hahn[23] obtained from crushed living tubercle bacilli a juice having the properties of a hydrolytic ferment. The tubercle bacillus has been found by de Schweinitz,[24] Koch and Proskauer,[4] and Unna[25] to contain considerable quantities of fat and cellulose, the former having the specific staining reaction. The whole subject evidently requires exhaustive study.

The experiments with dead tubercle bacilli and their extracts by Wyssokowicz,[26] Maffucci,[27] [28] Daremberg,[29] Prudden and Hodenpyl,[30] Koch,[6] Straus and Gamaleia,[31] Vissman,[32] Kostenitch,[33] Grancher and Martin,[34] Grancher and Ledoux-Lebard,[35] Freudenreich,[36] Masur and Kockel,[37] Abel,[38] Carriére,[39] Sciolla,[40] and Bâbes and Proca,[41] show their marked capacity to stimulate local cell-growths, to produce tubercles, local necrosis, aseptic abscesses, cachexia, with grave disturbance of the blood-forming functions, and nephritis. In the face of these facts it seemed hardly probable that the use of cultures containing tubercle bacillus, living or dead, would be practicable to create an increased tolerance. At least it appeared unlikely that animals would tolerate doses presumed to be necessary in order to originate antagonistic substances. That the tubercle bacillus substance is necessary to produce immunity was later claimed by Koch[4] in experiments with tuberculin "R."

We have endeavored to cover many but not all aspects of this subject in our experiments, and our methods have varied in some respects from those of other workers in this field whose developments we have followed. Without further discussing the theoretical considerations concerned, we will proceed to the description of our work.

The studies included in Part I. relate to the methods adopted by us in attempts at producing the sought-for immunity in various animals, and the tests of the germicidal and curative properties which might be possessed by the serum of animals thus immunized. The studies included in Part II. relate to tests in animals of the antitoxic power of serums in tuberculin poisoning.

Thus far we have employed sheep, fowls, asses, and rabbits in attempts to obtain antitoxic serum. Before giving details of the work the following summary will set forth the general methods employed by us to obtain the serums.

Part I.

1. Sheep repeatedly inoculated intravenously with filtrate of tubercle bacillus cultures on thymus bouillon.

2. Fowls repeatedly inoculated intraperitoneally with tubercle bacillus of mammalian tuberculosis of increasing virulence, and recovered.

3. Sheep injected subcutaneously with increasing doses of tuberculin.*

4. Sheep repeatedly inoculated intravenously with living non-virulent cultures of tubercle bacillus.†

5. Ass repeatedly inoculated intravenously with living non-virulent cultures of tubercle bacillus.

6. Ass inoculated: (*a*) Subcutaneously with virulent living cultures of tubercle bacillus; (*b*) intravenously with virulent tuberculous material, and recovered; (*c*) treated with tuberculin subcutaneously in increasing doses.

7. Ass injected: (*a*) Subcutaneously with dead cultures of non-virulent tubercle bacilli on thymus bouillon; (*b*) with precipitated tuberculin from proteid-free culture media of same non-virulent tubercle bacillus; (*c*) alkaline extracts of the bacilli with dead bacilli subcutaneously; (*d*) living non-virulent tubercle bacilli.

8. Rabbits: (*a*) Inoculated intravenously with non-virulent tubercle bacillus and recovered; (*b*) inoculated intraperitoneally with virulent tubercle bacilli and recovered.

With serums from the foregoing we tried to carry out the following series of tests:

1. Effect of serum on healthy animals.

2. Treatment of tuberculous animals with serums to show influence on course of disease and temperature.

3. Test of germicidal influence *in vivo* and *vitro*.

* The tuberculin used in this work was made from full-grown bouillon cultures of non-virulent tubercle bacillus, evaporated over water-bath to one-tenth volume and filtered through clay; 0.100 c.c. usually sufficed to kill guinea-pigs six weeks tuberculous.

† The cultures denoted "non-virulent" were from tubercle bacilli grown four years in the ncubator, and only occasionally killed guinea-pigs in six months to one year.

Part II.

Tests of antitoxic power of serums in tuberculin poisoning.

In these experiments we have considered:

1. Fatal doses of tuberculin* in sound animals.
2. Fatal doses in tuberculous animals.
3. Small doses in tuberculous animals to show effect: (*a*) On temperature; (*b*) local reaction.

We must acknowledge at the outset that for various reasons we were unable to carry out all of these tests with all of our serums, and the work is incomplete to that degree. Methods used in some tests were changed in others, because thought to be fallacious; particularly in testing for anti-tuberculin. Consequently there is no true basis for comparison for all the serums tried. In addition to the serums prepared by ourselves, we have tried five or six from other sources. In the present uncertain state of serum-therapy for tuberculosis it is undesirable to mention the names of their originators.

Many difficulties present themselves in any attempt to demonstrate a specific antitoxin in tuberculosis by methods analogous to those used so satisfactorily in diphtheria. The principal of these relate to the difficulty of obtaining a highly poisonous product in small bulk unless tuberculous instead of sound animals are employed for the tests.

Objections can easily be made to the use of tuberculin and diseased animals for testing serums. In the first place, tuberculin when prepared by boiling heat may not represent the unaltered toxins of tuberculosis, though containing more toxic extractive substances from the bacilli than when made by evaporation of cultures at low temperatures. Yet we cannot safely say that tuberculin is a wholly altered product until we have further knowledge of its chemical nature. We know, indeed, that boiling heat, not too prolonged, does not destroy its most prominent physiological action on tuberculous animals. The propriety of using tuberculin in serum tests principally depends upon the view one may take of the character of the peculiar reaction. That question may need further elucidation, but it seems to us that the best explanation we have from observations up to date supposes tuberculin to be a *partly specific irritant both to tuberculous foci and to*

* Under the term "tuberculin" we here include the various extracts of tubercle bacillus, but usually the original Koch fluid.

the susceptible organism in general. The local and general reaction is partly caused by the poisons contained in the tuberculin which irritates the sensitized cells composing the tubercle, and partly by those toxins set free by the hyperæmia or the enzyme action directly or indirectly produced by the dose of tuberculin (see Bâbes and Proca).[41] Whether the toxins liberated are the same, chemically, as those obtained from artificial cultures of tubercle bacillus, it is not easy to determine; but that some are set free seems obvious from the profound general disturbance brought about by a minute dose of tuberculin. According to this theory, the poisons are stored up in the tubercles, and in part, at least, derived from the dead or weakened bacilli, as shown by Bâbes and Proca[41] in experiments with dead tubercle bacilli. Crookshank and Herroun[11] and Matthes[19] have extracted albuminous poisons out of caseous material from tuberculous lesions which had the properties of tuberculin. Kahler[44] and Lenoir[45] found albumoses in urine of patients being treated with tuberculin, which produced tuberculin reactions and were present in the urine in excess of that injected as tuberculin. Thus we have some evidence of the discharge of accumulated pyrogenic substances, and that the tuberculin injected in the minute dose necessary to cause the reaction may not itself play the prominent part as a poison, but rather as the excitant to the discharge of other specific poison.

Therefore, but for the uncontrollable amounts of hoarded toxin, as it were, exploded, coupled with the local congestions, there would be no objection to proving the activity of serums in this way. Given the serum of a tuberculous animal having been treated with tuberculin, and tolerating the reactions well, we might hope, if an antitoxin had been in the serum, that it would be effective against the specific poisons developed in the animal body by tuberculosis, though not necessarily against tuberculin as obtained from cultures. By analogy, such an antitoxin ought also to manifest its activity on other tuberculous animals injected with a dose of tuberculin just large enough to barely produce a reaction (and, at the same time, with a dose of such serum sufficient to surely neutralize it). Should it be weak in activity it would still seem possible to obtain some effect in that way. Furthermore, it might reasonably be expected that the fatal effects of doses of tuberculin just sufficient to cause death in tuberculous animals with uniform lesions might be inhibited by the administration of an efficient

serum, or, at least, that the lives of the animals might be appreciably prolonged. Such tests ought to give qualitative results, though, from the unknown quantities of toxin to be dealt with, manifestly they would not serve as quantitative standards for serums.

On the other hand, healthy animals cannot be suitable for tests until we can obtain sufficiently toxic unaltered products of tubercle bacilli; while if the serums proved to be wholly or chiefly bactericidal and not antitoxic, they would not reveal that fact in non-tuberculous animals.

As is well known from Koch's original experiments, healthy guinea-pigs tolerate very large doses of tuberculin. This fact renders it necessary to employ such a quantity of ordinary tuberculin to produce death that the effects of the unaltered ingredients of the culture fluid, such as glycerin, albumoses, or peptone, should be remembered, as well as the shock produced by the traumatism of voluminous injections.

We occupied ourselves for some time in attempts to produce a concentrated tuberculin or toxin from cultures; but while it is comparatively easy to produce tuberculin, even from non-virulent cultures, which will usually be fatal in doses of 1 c.c. to 100 grammes of pig, the strength of toxin mentioned by Maragliano[46] as used to test his serum, yet this ratio did not suffice to kill pigs weighing above 250 grammes in our experiments. In addition to the objections mentioned, we found its absorption from under the skin very uncertain.

We then sought, by precipitation from cultures on proteid-free media, to obtain a pure toxin which would be fatal in small doses. We also attempted to procure a concentrated poison from the bacilli by using weak alkaline extracts, but none of our preparations were quickly fatal to healthy animals in small doses, though producing cachexia and death after some time. After testing various preparations subcutaneously and intraperitoneally without encouragement, we abandoned the idea of utilizing healthy animals for antitoxin or antituberculin tests. In the course of this work we had used fifty guinea-pigs, weighing from 150 to 450 grammes. More recently fresh extracts from crushed living tubercle bacilli gave no more uniform fatalities.

Clinical experience with human tuberculosis confirms the fact that the poisons are of relatively low toxicity—where their immediate effects are considered—as compared with those of diphtheria, tetanus,

etc. The same fact suggests itself as a reason for the indifferent results of experiments in serotherapy with tuberculosis. The effect of tuberculin administered to an animal after the disease becomes established is, nevertheless, so powerful and the dose so small that the objections mentioned do not enter.

A surely fatal dose of tuberculin for a tuberculous guinea-pig, though not uniform, is small enough for testing serums; yet there are other factors not to be disregarded in such experiments which are very difficult to control. The local reaction varies considerably in intensity in the same series of animals. Differences in virulence of the inoculation-material used, variations in lesions, extent of caseation, age, and pregnant state of animals, suffice to require large numbers of experimental animals in any work on tuberculosis. All these factors we found especially important in testing for antituberculin.

For the reasons mentioned, the fatal doses of tuberculin necessarily vary. Differences in stage of disease, the injection of serums mixed with tuberculin or given separately may produce diverse results, owing to different rate of absorption* or other reasons. We also found that non-tuberculous serums and even physiological salt solution, when used under like conditions, gave results that dampen over-enthusiasm in making deductions. Yet in the experiments that follow, in the course of which two hundred and fifty pigs were used, there can be seen indications of a favorable influence or possibly antitoxic effect from some of the serums on tuberculous guinea-pigs tested with fatal doses of tuberculin.

Details of Methods Used to Test Antitoxic Power of Serums.

The guinea-pigs were inoculated in lots of ten to twenty, at different times, with cultures or other tuberculous material of varying known virulence. The point of inoculation was usually below the right groin in the subcutaneous tissue.

The tuberculin used to kill varied in dose from 0.100 to 0.500 c.c., according to stage and type of disease in the animals, and was made

* The slow absorption of the infiltration produced by injections under the skin of guinea-pigs, of sheep, cow, ass, and rabbit serums, was a point specially noted by us in all this work, and has been of late particularly contrasted with the better absorption of horse serum by Uhlenhuth.[42]

from non-virulent bouillon cultures. The same bottle was used in nearly all the tests. For effect on temperatures, doses of 0.001 to 0.002 were employed.

The doses of serum ranged from 1 to 12 c.c. Some lots were tested with doses of serum and tuberculin proportioned to the weight of the pigs.

Loss of weight and strength were taken as the most practical criterions of condition of the diseased animals at the time of testing.

The tests were made from twenty-one to fifty-six days after infection.

To avoid false deductions from lack of absorption of tuberculin when mixed with serum, it was given separately in most of the experiments.

Serums were injected at the same time or before the tuberculin, subcutaneously, and in some instances intraperitoneally; the tuberculin always under the skin. Temperatures were taken in the rectum at the time of injection and six hours later. Some surviving animals were usually killed for comparison after twenty-four hours.

Normal horse serum, diphtheria antitoxin, normal sheep serum, 0.6 per cent. NaCl solution and hydrocele fluid were used to control anti-tuberculosis serums in comparative tests.

Résumé.

The results of our four-years' work in experiments upon four sheep, three asses, twelve fowls, eighteen rabbits, and four hundred and fifty guinea-pigs, are to be found in the following summary:

1. Sheep injected intravenously with killed thymus cultures: Result so unsatisfactory that the serum-tests were not conclusive of its value.

2. Chickens inoculated intraperitoneally with mammalian tuberculosis: Serum revealed no germicidal or inhibitive action on tubercle bacillus, nor favorable influence on the course of the disease in guinea-pigs.

3. Sheep injected with tuberculin: The serum was wanting in germicidal, antitoxic, or curative effect, so far as tested.

4. Sheep inoculated intravenously with non-virulent cultures: Cachexia followed, and, therefore, the serum was not used.

5. Ass inoculated as in 4; died from pulmonary embolus: Serum not bactericidal to tubercle bacillus.

6. Ass inoculated with virulent tubercle bacilli, treated with tuberculin and recovered: The serum showed no germicidal nor curative but possibly some antitoxic effect.

7. Ass inoculated with non-virulent tubercle bacilli and treated with various extracts of tubercle bacilli and dead bacilli: Serum showed no activity.

8. Rabbits inoculated with non-virulent and virulent tubercle bacilli and recovered: Serum possibly conferred some protection in tuberculin poisoning, and possibly prolonged lives of treated guinea-pigs.

Although we have a full appreciation of the uncertainty of correct conclusions from tests of the serums other than our own product, which were tried with tuberculin, only one indicated slight antitoxic power. This was obtained from a horse inoculated with non-virulent cultures.

That the apparent protection against fatal tuberculin poisoning occasionally seen was not necessarily from specific antitoxic power of the serum is made probable by the similar effects of physiological salt solution shown at times.

None of the serums appeared to prevent the local or general reaction from small doses of tuberculin, nor influence the temperature of tuberculous animals.

Disappointing as these results may seem, the writers feel that, in the light of recent contributions made by Ehrlich, Wasserman, and Behring[3] to our knowledge of the mechanism of immunity and antitoxin production in the body, the outlook for an efficient tuberculosis antitoxin is by no means a hopeless one.

In conclusion, we would express our thanks to Drs. S. W. Hewetson, W. S. Nelson, and J. A Wilder, all of whom have aided in the many arduous details of these experiments.

April 1, 1898. The full details of this work, more especially the experimental work which forms the basis of this paper, will be published in the *American Journal of the Medical Sciences*.

References.

1. Héricourt et Richet. De l'immunité conférée à des lapins, etc. Etudes exper. et clin., etc. Verneuil, 1888–90.

2. Trudeau, E. L. An Experimental Study in Preventive Inoculation in Tuberculosis. Medical Record, November 22, 1890.

3. Trudeau, E. L. Eye Tuberculosis and Antitubercular Inoculation in the Rabbit. New York Medical Journal, July 4, 1893.

4. Koch, R. Ueber neue Tuberculin prepärata. Deutsch. med. Wochen., No. 14, 1897.

5. Hammerschlag. Bacteriologisch-chemische Untersuchungen über Tuberkelbacillen. Central. f. klin. Med., 1892, p. 9.

6. Koch, R. Weitere Mittheilung über das Tuberkulin. Deutsch. med. Wochen., No. 43, 1891.

7. Proskauer u. Brieger. (Ref. by Koch. Analysis of Tuberculin.) Deutsch. med. Wochen., No. 43, 1891.

8. Hueppe, F., u. Scholl, H. Ueber die Natur der Koch'schen Lymph. Berlin. klin. Wochen., Nos. 4–8, 1891.

9. Weyl, Th. Zur Chemie und Toxikologie des Tuberkelbacillus. Deutsch. med. Wochen., No. 7, 1891.

10. Héricourt et Richet. De la toxicité des substances solubles des cultures tuberculeuses. Compt.-Rend. Hebd. Soc. de Biol., No. 21, 1891.

11. Crookshank, E. M., and Herroun, E. F. On the Chemical Properties and Physiological Effects of the Products of the Tubercle Bacillus in Pure Cultivation. British Medical Journal, February 21, 1891.

12. Richet. Ch. De la toxicité des substances solubles des cultures tuberculeuses. La Semaine Méd., No. 31, 1894.

13. Bäbes, A. et V. Sur certaines substances chimiques produites par le bacille de la tuberculose et sur tuberculose aviaire. Congrès p. Tuberculose, 1891.

14. Zuelzer. Ueber ein Alkaloid der Tuberkelbacillen. Berlin. klin. Wochen., No. 4, 1891.

15. Klebs E. Die Causale Behandlung der Tuberkulose. Hamburg, 1894.

16. Hahn, Martin. Ueber die Chemische Natur des wirksamen Stoffes in Koch'schen Tuberculin. Berlin. klin. Wochen., No. 30, 1891.

17. Kühne, W. Erfahrungen über Albumosen und Peptone. Zeitsch. f. Biologie, Bd. xi. 1, xii. 2.

18. Hoffman, R. R. Zur Kenntniss der Eiweisskörper in dem Tuberkelbacillen. Wiener klin. Wochen., No. 38, 1894.

19. Matthes, M. Ueber die Wirkung einiger subcutaneinverleibter Albumosen, etc. Deutsch. Archiv f. klin. Med., 1894.

20. de Schweinitz, E. A., and Dorset, M., et al. Tuberculosis Investigations. Bull. U. S. Bureau of Animal Industry, Nos. 7 and 13.

21. Behring, E. Ueber experimentelle begründete aetiologische Therapie. XV. Cong. f. innere Med., Berlin, 1897.

22. de Schweinitz, E. A. Some Products of the Tuberculosis Bacillus and the Treatment of Experimental Tuberculosis with Antitoxic Serum. Transactions of the Association of American Physicians, 1897.

23. Hahn, Martin. Immunisirungs und Heilversuche mit den plasmatischen Zellsäften von Bacterien. München. med. Wochen., No. 48, 1897.

24. de Schweinitz, E. A., and Dorset, M. The Composition of the Tuberculosis and Glanders Bacilli. Journal Amer. Chem. Soc., August, 1895; March, 1896.

25. Unna. Feltgehalt de Tuberkel und Leprabacillen. (Abstr.) München. med. Wochen., No. 5, 1897.

26. Wyssokowicz. (Injections of Dead Bacilli. Ref. Masur et Kockel.) Separat-ab. aus Mittheil. aus Dr. Brehmer's Heilanstalt, etc. Wiesbaden, 1890.

27. Maffucci, A. Ueber die Wirkung der reinen, sterilen Culturen des Tuberkelbacillus. Central. f. allg. Path. u. path. Anat., No. 26, 1890.

28. Maffucci, A. Ueber die Wirkung der Produkte des Tuberkelbacillus. Deutsch. med. Zeitung, No. 24, 1892.

29. Daremberg, G. Reaction des subjets tuberculeux sous l'influence des liquides de l'organism sain. Compt.-Rend. Hebd. Soc. de Biol., vol. v., December 30, 1893.

30. Prudden, T. M., and Hodenpyl, E. Studies on the Action of Dead Bacteria in the Living Body. New York Medical Journal, June 6 and 20, 1891.

31. Straus, J., et Gamaléia, N. Recherches expérimentales sur la tuberculose, etc. Arch. méd. Exp. d'Anat. Path., vol. iii., No. 4, 1891; Etudes, etc., sur la Tuberculose, vol. iii. p. 433.

32. Vissman, W. Wirkung todter Tuberkelbacillen und des Tuberkulins auf den Thierschen Organismus. (Thesis, Berlin, 1892.)

33. Kostenitsch, J. De l'évolution de la tuberculose provoquée chez les lapins par les bacille morts, etc. Arch. méd. Exp. d'Anat. Path., vol. v., No. 1, 1893.

34. Grancher, J., et Martin, H. Notes sur les vaccinations antituberculeuses. Congrès p. Tuberculose, 1891, 1896.

35. Grancher, J., et Ledoux-Lebard. Etudes sur la tuberculeux expérimentale du lapin. Arch. de méd. Exper. d'Anat. Path., No. 2, 1891.

36. Freudenreich, E. V. Ueber die toxische Wirkung der Cultur products den Hühnertuberculose. (Ref. Baumgarten's Jahresb., 1893.)

37. Masur, A., u. Kockel. Zur Kenntniss von der Wirkung todted Tuberkelbacillen. Ziegler's Beiträge, vol. xvi., 2, 1894.

38. Abel. (Injections of Dead Tubercle Bacilli.) Deutsch. med. Wochen., p. 482, 1892. (Ref. Masur u. Kockel.)

39. Carrière, G. Etudes expérimentales des altérations histologiques du foie et du rein produites par les toxines tuberculeuse (tuberculin). Arch. de méd. Exper. et d'Anat. Path., vol. ix., No. 1, January, 1897.

40. Sciolla. (Die toxine der Tuberculose.) VIII. Cong. Rome, October, 1896. (Abstr.) Hygienische Rundschau, vol. vi., No. 17, 1897.

41. Bäbes, V., u. Proca, G. Untersuchungen über die Wirkung der Tuberkelbacillen und über gegenwirkender Substanzen. Zeitsch. f. Hygien. u. Infekt., vol. xxiii., No. 3, 1896.

42. Uhlenhuth. Zur Kenntniss der giftigen Eigenschaften der Blutserums. Zeitsch. f. Hygiene u. Infekt., vol. xxvi., No. 3, 1897.

43. Behring, E. Ueber Heilprincipien, insbesondere über das aetiologische und des isopathische Heilprincip. Deutsch. med. Wochen., No. 5, 1898.

44. Kahler. Peptonurie nach Injektion des Koch'schen Mittels. Wien. klin. Wochen., No. 3, 1891.

45. Lenoir. (Albumoses in Urine of Tuberculous Patients.) Ref. Matthes. Progrès Méd., No. 30, 1893.

46. Maragliano. Le sérum antituberculeux et son antitoxine. Revue de la Tuberculose. No. 2, 1896.